Effect of planting density on plant growth and camptothecin content of *Camptotheca acuminata* seedlings

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Abstract: *C. acuminata* seedlings cultivated in greenhouse were transplanted into the fields with 5 designed planting densities (11, 16, 25, 44 and 100 plants·m^{-2}) in May of 2004 and were harvested in the middle of September of 2004. The seedling growth indexes including plant height and crown width, biomass allocation, camptothecin (CPT) content and CPT yield of different organs (young leaf, old leaf, stem, and root) were studied. For the 5 selected planting densities, the plant biomass, height, crown width, and total leaf area of *C. acuminata* seedlings all showed highest values at the planting density of 25 plants·m^{-2}. CPT content in young leaves was higher than that in other organs of seedlings and presented an obvious change with the variation of planting densities and with the highest value at density of 100 plants·m^{-2}, while for other organs no significant variation in CPT content was found with change of planting density. The accumulation of CPT was enhanced significantly at the planting density of 25 plants·m^{-2}. It is concluded that for the purpose to get raw materials with more CPT from *C. acuminata*, the optimal planting density of *C. acuminata* seedlings should be designed as 25 plants·m^{-2}.

Keywords: *Camptotheca acuminata* seedlings; Planting density; Biomass; Camptothecin

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Introduction

Planting density has been proved to be a significant factor affecting plant growth. Fang (2004) observed that there were significant differences in above-ground biomass, bark yield and bark ratio of *Pteroceltis tatarinowii* among different planting densities. Increasing planting density affected root and fruit growth per plant but increased yield of *Pteroceltis tatarinowii* to a certain extent (Leidi 2004). A 6-year study showed that planting density had evident effect on River red gum (*Eucalyptus camaldulensis*) growth. Tree size was greatest in the wide spacing of the lower planting density. Per acre volume and weight yields were greater at the higher planting density, while the height, diameter, volume and the weight of individual tree were greater at the low planting density (Cockerham 2004). However, very few reports were found as concerning the effect of planting density on secondary metabolites in plants.

Camptotheca acuminata Descne, a native tree of China, is extensively distributed in the Changjiang Valley. Camptothecin (CPT), an alkaloid with high anti-cancer activity in *C. acuminata* (Wall 1966), is an active composition exclusively inhibited topoisomerase I (topo I), (Hsiang 1985). Though CPT was toxic and has no clinical application value, some CPT derivatives with the same anti-cancer function and efficiently low toxicity had been used on clinic, such as topotecan and CPT-11 which were approved by the Food and Drug Administration (FDA), USA in the treatment colon/rectum cancer and the ovary cancer (Potmesil 2000), in addition, several others were still under clinical trials.

The objective of this study is to investigate the effect of planting density on CPT content and tree growth. We determined CPT content and measured different plant growth indices of 5 planting densities.

Materials and methods

Seedling cultivation and treatments

At the beginning of May of 2004, seedlings from *C. acuminata* seeds were grown in the greenhouse. When three pairs of real leaves grew out, the seedlings were transplanted into the field and planted in 5 different plant and row spacing (10, 15, 20, 25, and 30 cm), equivalent to the planting densities of 100, 44, 25, 16 and 11 plants·m^{-2}, respectively. Normal field management was preformed during the time course of treatment. The seedlings were harvested in the middle of September.

Analysis of seedling growth and CPT contents

On September 15, 2004, 10 seedlings, randomly selected, were measured for the plant height and crown width, and they were harvested after the measurements. According to the method of Yan (2003), young leaf was identified as any leaf that located from the apex down to the largest leaf in the exact branch, otherwise it was old leaf. The seedlings were divided into four-parts, i.e. young leaves, old leaves, stems and roots.

Leaf area was measured by LI-3000A potable leaf area meter (LI-COR Co., USA). Young leaves, old leaves, stems, and roots were dried to constant weight and weighed, after then they were ground to 60 mesh and conserved for the determination of CPT content.

Following Yan's method (2002), CPT content was determined by Waters HPLC with 2996 diodearray detector (Waters Co., USA). CPT content was expressed as mg/g dry weight (DW). The yield of CPT was calculated by the product of CPT content and the biomass of *C. acuminata* seedlings. All data were analyzed by using DPS (Tang 2002).

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Results and discussion

Effect of planting density on *C. acuminata* seedling growth

It was observed that planting density had remarkable effect on seedling growth of *C. acuminata*. The height of *C. acuminata* seedling increased with increase of planting density to a certain extent. The most favorable panting density for both height growth and crown width was 25 plants · m^{-2} (Fig. 1 and Fig. 2).

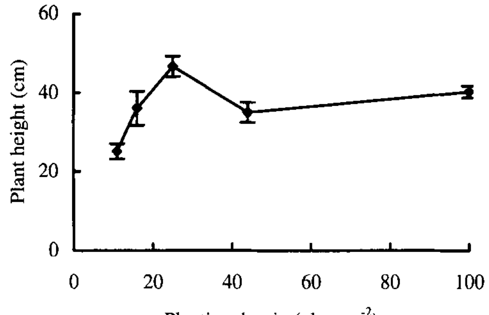

Fig. 1 Effect of planting density on height growth of *C. acuminata* seedlings

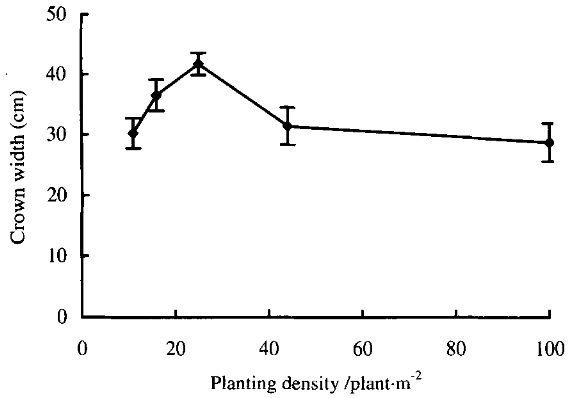

Fig. 2 Effect of planting density on crown width of *C. acuminata* seedlings

The different planting densities led to a variety of plant biomass. The biomasses of root, leaf and the whole-plant were the highest at the planting density of 25 plants · m^{-2}, and lowest at that of 100 plants · m^{-2}, whereas the stem biomass was higher at the planting density of 100 plants · m^{-2} than that of 11 plants · m^{-2} (Fig. 3). The variations in old leaves had the same biomass regulation as those in young leaves (Fig.4). All these results indicated that the optimal planting density for *C. acuminata* seedlings growth was 25 plants · m^{-2}.

Biomass allocation in *C. acuminata* seedlings was relatively stable. The biomass of leaf accounted for 52%–55% of total biomass, which was higher than that of root and stem significantly (Fig 5). With planting density increasing, the percentage of stem biomass increased, while that of root biomass showed a decreased trend (Fig.5). Increasing planting density lead to growth competition for achieving more spacing, which inhibits underground growth but enhances the stem growth (Degenhardt 1981; Rao 1989; Morris-son 1990).

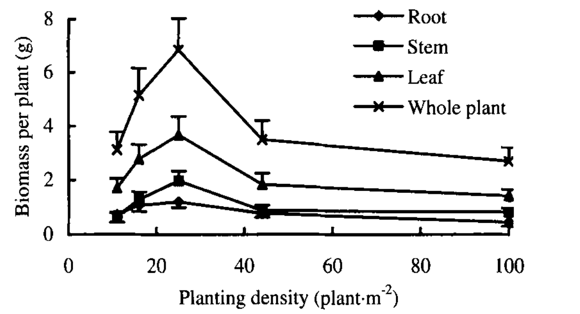

Fig. 3 Effect of planting density on biomass in *C. acuminata* seedlings

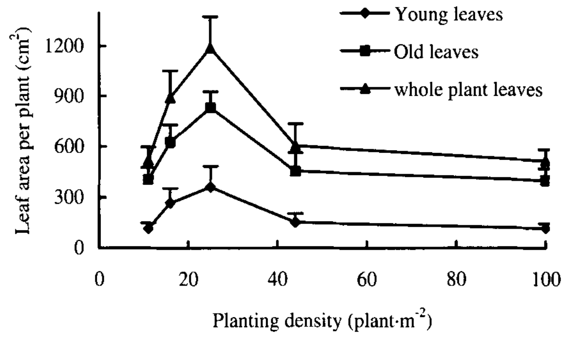

Fig. 4 Effect of planting density on leaf area in *C. acuminata* seelings

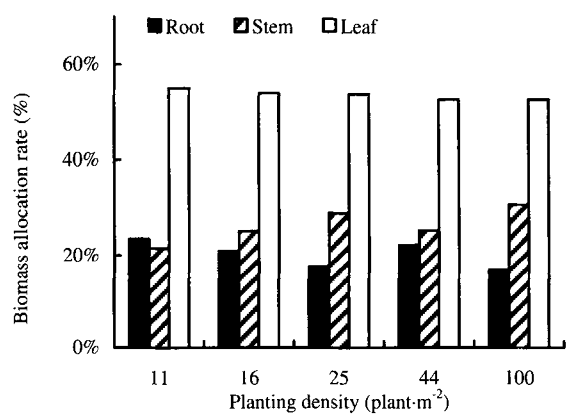

Fig. 5 Effect of planting density on biomass allocation in *C. acumnata* seedlings

Effect of planting density on CPT content in *C. acuminata* seedlings

CPT contents in different organs of *C. acuminata* seedlings were different. Young leaves had highest content of CPT, followed by old leaves, and the content of CPT in stem or root was very low. CPT content in young leaves positively related to the planting density and the highest content (0.73 mg/g DW) occurred at the planting density of 100 plants · m^{-2}; CPT content in old leaves also positively related to planting density, but the difference was not significant among the different densities. For CPT contents in stem and foot, the lowest value were found at the planting density of 11 plants · m^{-2}, but no significant variation was observed with change of planting density (Fig.6).

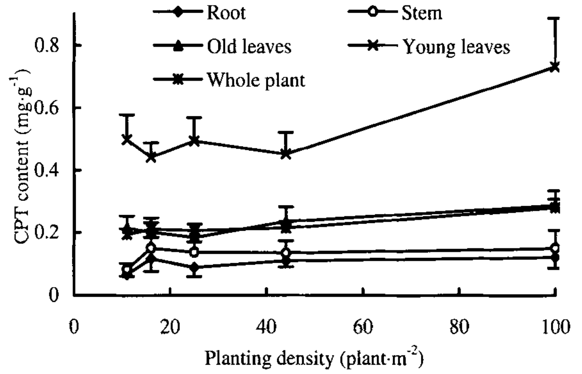

Fig. 6 Variation of camptothecin (CPT) contents in different organs of *C. acuminata* seedlings at different planting densities

Variation of CPT yield in *C. acuminata* seedlings

The yield of CPT was calculated by the product of CPT content and the biomass of *C. acuminata* seedlings to explored the total CPT accumulation in the seedlings or organs.

CPT yields from different organs of *C. acuminata* seedlings showed a similar correlation with planting densities. Except the root, the CPT yield was highest at the planting density of 25 plants · m^{-2}, and the total CPT yield of whole seedling at this density was much higher than those at other densities (Fig. 7). The CPT yields for all the organs had little change when the planting density was over 44 plants · m^{-2}.

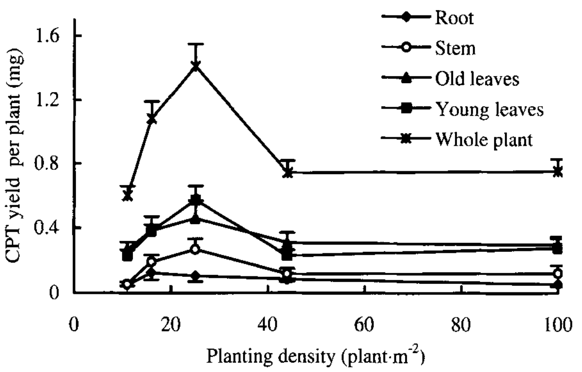

Fig. 7 Variation of camptothecin (CPT) yields from different organs of *Camptotheca acuminata* seedlings at different planting densities

Conclusions

For the 5 selected planting densities (11, 16, 25, 44, and 100 plants · m^{-2}), the plant biomass, height, crown width, and total leaf area of *C. acuminata* seedlings all showed highest values at the planting density of 25 plants · m^{-2}, that is to say the optimal planting density for seedling growth of *C. acuminata* was 25 plants · m^{-2}.

CPT content in young leaves changed obviously with the changes of planting densities, and high planting density could increase CPT contents in leaves effectively.

Under the condition of selected planting densities, the accumulation of CPT was enhanced significantly at the planting density of 25 plants · m^{-2}. For the purpose to get raw materials with more CPT from *C. acuminata*, the optimal planting density of *C. acuminata* seedlings should be designed as 25 plants · m^{-2}.

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